




A surge in serum mucosal cytokines associated with seroconversion in children at risk for type 1 diabetes

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Keywords

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ABSTRACT

Aims/Introduction: Autoantibodies to pancreatic islet antigens identify young children at high risk of type 1 diabetes. On a background of genetic susceptibility, islet autoimmunity is thought to be driven by environmental factors, of which enteric viruses are prime candidates. We sought evidence for enteric pathology in children genetically at risk for type 1 diabetes followed from birth who had developed islet autoantibodies ("seroconverted"), by measuring mucosa-associated cytokines in their sera.

Materials and Methods: Sera were collected 3 monthly from birth from children with a first-degree type 1 diabetes relative, in the Environmental Determinants of Islet Autoimmunity (ENDIA) study. Children who seroconverted were matched for sex, age, and sample availability with seronegative children. Luminex xMap technology was used to measure serum cytokines.

Results: Of eight children who seroconverted, for whom serum samples were available at least 6 months before and after seroconversion, the serum concentrations of mucosa-associated cytokines IL-21, IL-22, IL-25, and IL-10, the Th17-related cytokines IL-17F and IL-23, as well as IL-33, IFN- γ , and IL-4, peaked from a low baseline in seven around the time of seroconversion and in one preceding seroconversion. These changes were not detected in eight sex- and age-matched seronegative controls, or in a separate cohort of 11 unmatched seronegative children.

Conclusions: In a cohort of children at risk for type 1 diabetes followed from birth, a transient, systemic increase in mucosa-associated cytokines around the time of seroconversion lends support to the view that mucosal infection, e.g., by an enteric virus, may drive the development of islet autoimmunity.

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INTRODUCTION

Environmental factors acting on a background of genetic susceptibility are believed to drive pancreatic islet autoimmunity, leading to the destruction of insulin-secreting beta cells in type 1 diabetes. Circulating autoantibodies to islet antigens are currently the best-documented marker of sub-clinical (stage 1) type 1 diabetes. Most children who develop clinical (stage 3) disease display autoantibodies to one or more of the islet antigens insulin, glutamic acid decarboxylase 65 [GAD], islet-associated antigen-2 [IA-2], and zinc transporter 8 [ZnT8], by 5 years of age^{1,2}. The environmental factors that promote the development of islet autoimmunity remain poorly defined, but enteric viruses are considered prime candidates^{3–5}. We surmised that if enteric or indeed other mucosal infection promoted islet autoimmunity, then the appearance of islet autoantibodies (“seroconversion”) may be associated with circulating mucosa- and Th17-related cytokines, reflecting mucosal inflammation. The Environmental Determinants of Islet Autoimmunity (ENDIA) study⁶, in which children with a first-degree relative with type 1 diabetes are followed from pregnancy through early life, provided the opportunity to examine this hypothesis.

MATERIALS AND METHODS

Study protocol

Caucasian children with a first-degree relative with type 1 diabetes participated in the multi-site Australian Environmental Determinants of Islet Autoimmunity (ENDIA) pregnancy-birth cohort study⁶. Recruitment to ENDIA commenced in February 2013 and closed in December 2019. The primary endpoint of ENDIA is seroconversion to islet autoimmunity, defined as the detection of islet autoantibodies in two or more consecutive blood tests taken at least 3 months apart. Venous blood is collected from children 3 monthly from birth to 2 years of age, and 6 monthly thereafter, until 10 years of age, or until the development of clinical type 1 diabetes as defined by the commencement of insulin therapy. Serum samples were frozen at –80°C until analyzed.

The analyses described herein were undertaken with samples and data collected up to the first quarter of 2018 when 1,050 dyads were enrolled, and 970 babies had been born. By this time, 23 children had seroconverted with at least one persisting islet autoantibody. The investigation of a possible association between seroconversion and serum cytokines necessitated the availability of sequential serum samples in the 6 months before and after seroconversion. However, because of missed visits or failure to gain venous access, sample collections in the months before and after seroconversion were not complete and only eight of the 23 children met these criteria. These did not differ in median age, sex ratio, or number of autoantibody specificities from the 23 in total. Each of the eight seroconverters (cases) was matched for birth sex and age (birth date ± 45 days) to a seronegative control who had similarly available serial serum samples. The smallest date of birth gap between

Table 1 | Characteristics of study participants

Characteristic	Case (n = 8)	Control (n = 8)
Male, n (%)	3 (38)	3 (38)
Female, n (%)	5 (63)	5 (63)
HLA-DR, n (%)		
3, 4	4 (50)	2 (25)
3, 3	1 (13)	0
4, 4	0	0
3, X	0	1 (13)
4, X	2 (25)	3 (38)
X, X	1 (13)	2 (25)
Relative with type 1 diabetes, n (%)		
Mother	3 (38)	5 (63)
Mother and sibling	0	1 (13)
Father	1 (13)	0
Sibling	4 (50)	2 (25)
Single Ab at seroconversion, n (%)	5 (63)	–
Multiple Ab at seroconversion, n (%)	3 (38)	–
First autoantibody at seroconversion, n (%)		
IAA	3 (38)	–
GAD	2 (25)	–
ZnT8	1 (13)	–
IA2A	0	–
IAA, GAD	1 (13)	–
IAA, GAD, ZnT8	1 (13)	–
Progression to clinical type 1 diabetes	4 (50)	0
Mean age in months (SD) at diagnosis of type 1 diabetes	53.7 (26.0)	–
Mode of delivery		
Vaginal, n (%)	3 (38)	4 (50)
Caesarean with labor, n (%)	1 (13)	0
Caesarean without labor, n (%)	4 (50)	4 (50)
Infant feeding		
Breastfed, n (%)	6 (75)	8 (100)
Missing data, n (%)	2 (25)	0
Mean age (months) at which exclusive breastfeeding ended	2.2	1.7
Mean age (months) at introduction of solids	4.4	5.0
Immunizations complete in first year of life, n (%)	5 (63)	8 (100)
Missing data, n (%)	3 (38)	0

the pairs was 3 days and the largest 33 days; the median gap between pairs was 24 days. The characteristics of the cases and controls are shown in Table 1. In addition, serum cytokines were measured serially in a further 11 unmatched seronegative children recruited across the same time period as the cases.

A difference in the serum concentration of cytokines was based on comparison of the maximum value (peak) to the value at the preceding collection time point (pre) of cases (Table 2). For controls, the same time points as for cases were used unless there was no matching time point, whereupon the closest matching time point was used. Seroconversion was

Table 2 | Serum cytokine concentrations (pg/mL) in cases and controls

	IL-1 β (LOD 0.02)		IL-4 (LOD 0.52)		IL-6 (LOD 0.67)		IL-10 (LOD 0.3)		IL-17A (LOD 0.49)		IL-17F (LOD 0.8)		IL-21 (LOD 2.13)	
	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak
Case 1	0.34	0.34	1.5	44.8	1.6	26.2	2.1	35.2	2.26	2.3	13.5	190	127	692
Case 2	6.92	2.00	1.5	63.1	49.9	31.8	13.6	34.4	1.76	1.8	11.8	75	15	15
Case 3	0.34	0.34	9.8	42.2	1.6	20.0	8.7	42.4	2.26	2.3	26.7	220	127	1,665
Case 4	0.34	0.34	1.5	37.0	1.6	1.6	2.1	16.8	2.26	2.3	18.1	164	127	809
Case 5	0.34	0.34	20.0	56.4	9.4	25.2	8.7	30.3	2.26	2.3	88.8	283	127	1,015
Case 6	0.34	0.34	7.8	77.3	1.6	41.8	2.1	49.5	2.26	2.3	15.8	500	127	1,295
Case 7	0.34	0.34	14.7	26.8	7.5	11.9	2.1	16.5	2.26	2.3	30.9	106	127	736
Case 8	0.34	4.68	58.1	301	22.2	358	47.7	258	2.26	10.9	192	2,052	1,606	17,395
Case: back transformed mean (95%CI)	0.50 (0.28, 0.87)	0.59 (0.34, 1.03)	6.7 (3.1, 14.6)	59.4 (27.6, 128)	5.17 (2.32, 11.5)	24.3 (10.9, 54.1)	5.59 (2.70, 11.6)	39.1 (18.9, 81.0)	2.19 (1.77, 2.72)	2.70 (2.18, 3.36)	29.9 (16.6, 53.8)	251 (140, 452)	134 (56.4, 316)	835 (353, 1,976)
Tukey group+	–	–	B	A	B	A	B	A	–	–	B	A	B	A
Control 1	1.24	1.12	1.48	25.2	5.48	9.72	1.52	12.8	1.76	1.76	2.94	18.8	15.4	15.4
Control 2	0.92	1.24	1.48	1.48	4.04	10.1	9.08	1.52	1.76	1.76	2.94	2.94	15.4	15.4
Control 3	1.12	1.12	16.9	1.48	17.1	15.7	7.12	9.08	1.76	1.76	2.94	2.94	15.4	15.4
Control 4	1.12	0.92	1.48	1.48	4.56	4.20	13.6	1.52	1.76	1.76	2.94	2.94	15.4	15.4
Control 5	1.12	1.04	1.48	1.48	5.48	5.84	1.52	1.52	1.76	1.76	2.94	2.94	15.4	15.4
Control 6	1.24	NA	1.48	NA	4.76	NA	7.12	NA	1.76	NA	2.94	NA	15.4	NA
Control 7	0.80	1.12	1.48	1.48	8.32	5.48	1.52	1.52	1.76	1.76	2.94	2.94	15.4	15.4
Control 8	1.12	1.12	1.48	1.48	13.2	6.20	8.12	1.52	1.76	1.76	2.94	2.94	15.4	15.4
Control: back transformed mean (95%CI)	1.07 (0.61, 1.89)	1.08 (0.60, 1.96)	2.0 (0.9, 4.3)	2.2 (1.0, 5.0)	6.87 (3.08, 15.3)	7.30 (3.12, 17.2)	4.53 (2.19, 9.38)	2.65 (1.22, 5.75)	1.76 (1.42, 2.18)	1.75 (1.40, 2.22)	2.94 (1.63, 5.29)	3.83 (2.05, 7.17)	15.4 (6.51, 36.5)	15.6 (6.25, 38.7)
Tukey group+	–	–	B	B	AB	AB	B	B	–	–	C	C	C	C
Interaction P-Value	0.74		0.006		0.03		0.0002		0.31		0.0005		0.01	

+ Letters for pair-wise comparisons between means (if appropriate) of same cytokine. LOD, limit of detection in pg/mL; NA, serum sample not available.

defined as the appearance of one or more confirmed and persisting serum autoantibodies to insulin, GAD, IA-2, or ZnT8.

Health events data of children were documented weekly in infant feeding diaries completed by parents/guardians during the first year of life and at 3 monthly study visits until 2 years of age, and 6 monthly thereafter. All reported illnesses and symptoms were extracted and classified into World Health Organization's International Classification of Diseases (ICD-11) codes⁷. These codes were grouped into infection categories including respiratory and gastrointestinal infections, adapted from the TEDDY protocol⁸.

Islet autoantibody assays

Autoantibodies to insulin (IAA) were measured by immunoprecipitation of ¹²⁵I-human insulin, corrected for non-specific binding in the presence of excess unlabeled insulin. In the Islet Autoantibody Standardization Program (IASP) 2020 Workshop, this assay had a sensitivity of 36% and a specificity of 100%.

The threshold for positivity was IAA >0.7 Units. Autoantibodies to GAD (GADA), IA2 (IA2A) and ZnT8 (ZnT8A) were measured by immunoprecipitation of ³⁵S-methionine-labeled recombinant human proteins. GADA, IA2A, and ZnT8A assays scored 52, 64, and 52% for sensitivity and 100, 100, and 98% for specificity, respectively, in the IASP 2016 Workshop. The results were expressed in arbitrary units in comparison with positive and negative controls, with positivity defined as GADA >5.0 Units, IA2A >3.0 Units, and ZnT8A >3.1 Units. Positive results were confirmed by repeat testing.

Cytokine assays

Cytokines in the Human Th17 Cytokine Panel (listed in Table 2) were measured using Luminex xMAP technology on the Bio-Plex 200 platform and analyzed with Bio-Plex Manager 5.0 software (Bio-Rad Laboratories Ltd, Hercules, CA). Serially diluted standards and test sera diluted 1 in 4 in sample diluent were added in 50 μ L to a plate containing biotin-labeled

IL-22 (LOD 0.3)		IL-23 (LOD 1.55)		IL-25 (LOD 0.07)		IL-31 (LOD 0.49)		IL-33 (LOD 0.58)		IFN- γ (LOD 0.43)		TNF- α (LOD 0.07)		sCD40L (LOD 0.41)	
Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak
5.33	34.6	26	797	2.18	13.6	379	426	9.4	115	2.6	104	14.4	22.4	448	525
4.33	4.3	20	400	1.20	6.8	430	329	19.6	145	2.2	54	53.8	33.1	1,596	1,209
5.33	48.1	144	1,007	2.18	20.8	57.8	292	9.4	149	19.9	137	13.7	13.7	276	269
5.33	5.3	131	1,260	2.18	2.2	356	426	9.4	44.3	13.1	97	11.6	18.5	343	601
5.33	31.7	427	1,179	2.18	20.8	57.8	555	63.8	143	59.9	141	10.1	29.3	135	593
5.33	58.2	139	2,222	2.18	38.7	57.8	426	9.4	257	13.1	239	15.0	29.6	221	476
5.33	5.3	244	502	2.18	9.5	461	333	40.0	73.3	22.6	49	18.6	34.8	740	412
88.2	458	1,063	13,515	16.4	426	579	1,331	155	1,928	138	1,258	22.0	83.1	606	1,305
7.38	25.3	140	1,279	2.60	18.5	203	454	22.3	166	15.7	141	17.2	28.8	416	594
(3.64, 14.9)	(12.5, 51.2)	(66.7, 295)	(608, 2,695)	(1.37, 4.50)	(9.73, 35.2)	(114, 365)	(254, 814)	(11.2, 44.3)	(83.3, 330)	(6.93, 35.5)	(62.2, 318)	(12.2, 24.2)	(20.4, 40.5)	(261, 665)	(372, 949)
B	A	B	A	B	A	—	—	B	A	B	A	A	A	—	—
4.33	4.33	20.2	20.2	1.20	1.20	292	366	4.4	33.7	16.9	25.9	35.4	36.8	1,643	1,431
4.33	4.33	20.2	98.4	1.20	1.20	20	123	4.4	4.4	2.20	3.64	20.2	18.1	289	537
4.33	4.33	144	20.2	1.20	1.20	292	142	27.9	4.4	2.20	2.20	31.2	23.3	1,469	489
4.33	4.33	98.4	20.2	1.20	1.20	142	180	4.4	4.4	2.20	2.20	11.5	10.3	588	1,007
4.33	4.33	20.2	20.2	1.20	1.20	218	255	4.4	4.4	2.20	2.20	35.0	25.3	634	1,062
4.33	NA	20.2	NA	1.20	NA	85	NA	4.4	NA	2.20	NA	27.3	NA	400	NA
4.33	4.33	20.2	20.2	1.20	1.20	104	123	4.4	4.4	2.20	2.20	30.2	27.3	680	400
4.33	4.33	20.2	20.2	1.20	1.20	311	384	4.4	4.4	3.64	28.6	26.3	25.3	1,485	1,618
4.33	4.38	31.5	25.3	1.20	1.21	140	195	5.54	5.88	3.02	4.85	25.8	22.3	754	812
(2.14, 8.76)	(2.07, 9.25)	(14.9, 66.3)	(11.4, 56.2)	(0.63, 2.28)	(0.61, 2.41)	(78.0, 250)	(105, 363)	(2.79, 11.0)	(2.82, 12.3)	(1.34, 6.84)	(2.04, 11.5)	(18.3, 32.1)	(15.3, 32.1)	(472, 1,204)	(493, 1,340)
B	B	C	C	B	B	—	—	C	BC	C	BC	A	A	—	—
0.04		0.0007		0.0005		0.36		0.004		0.01		0.03		0.52	

antibody-coupled magnetic beads for each of the 15 cytokines in the panel. Samples were incubated at room temperature on a plate shaker at 300 rpm for 30 min. Following washing, secondary detection antibodies were added in 25 μ L to each well and incubated as described above. After a further wash, streptavidin-phycoerythrin (streptavidin-PE) was added in 50 μ L, and the plate incubated at room temperature on an orbital shaker at 300 rpm for 10 min. Assay buffer (125 μ L) was added to each well before analysis on the Bio-Plex 200 suspension array system. Fluorescent intensities obtained for the test samples were converted to pg/mL using the standard curves for each cytokine. Out-of-range cytokine concentrations were assigned a value corresponding to the minimum or maximum detectable concentration of the standard. The lower limit of detection varied for each cytokine (Table 2). According to the manufacturer's specifications, the intra- and inter-assay coefficients of variation were <10 and <20%, respectively, for all cytokines.

Statistical analysis

For each cytokine response, a separate linear mixed model was fitted with fixed factors *status* (case vs control), *time* (pre and peak values of cases), their interaction, and a random factor for matching groups which had eight levels corresponding to each of the eight matched case and control pairs. Of interest is the interaction between status and time, as a significant term implies that the serum cytokine concentration pre vs peak is different depending on case vs control status. Models were fitted using the package lme4 for statistical software platform R v4.1.1⁹. A log transformation of the cytokine response was employed to meet the underlying model assumptions. Back transformation of predicted values and standard errors of transformed responses was undertaken¹⁰. Back-transformed means (original scale) are shown (Table 2). A significance level of 5% was taken for fixed effects with type II testing conducted using Wald's test. When a significant interaction between status and time was found, to compare pairs of means a Tukey's

adjustment to the significance level was made to maintain an overall family-wise significance level of 5%.

RESULTS

The relationships between serum cytokine concentrations, age, and islet autoantibodies in cases and matched controls are depicted in Figure 1a,b and pre and peak serum cytokine concentrations are documented in Table 2. Serum mucosa-associated cytokines increased in all seropositive cases but not in the matched seronegative controls. When the pre and peak concentrations were compared, nine cytokines exhibited a significant interaction between status (case *vs* control) and time (pre *vs* peak) (Table 2). For IL-22, IL-25, IL-33, IFN- γ , IL-4, and IL-10, the peak values in cases were significantly different from both pre and peak values in controls; for IL-21, IL-23, and IL-17F, both pre and peak values in cases were significantly different from pre and peak values in controls. In 7/8 cases, serum cytokines peaked around the time of appearance of islet autoantibodies, and in one case the peak preceded seroconversion. To reinforce the association between the cytokine surge and seroconversion, serum cytokines were measured serially in a further 11 unmatched, seronegative children (Table S1). No child in this validation control cohort exhibited a cytokine surge.

Parent-reported intestinal or respiratory infections showed no obvious relationship to either the serum cytokine surge or seroconversion.

DISCUSSION

In a small cohort of children at increased risk for type 1 diabetes, we observed a transient surge in the serum concentrations of specific cytokines known to be associated with mucosal pathology and implicated in the pathogenesis of type 1 diabetes. Age- and sex-matched seronegative controls, and a separate cohort of unmatched seronegative children, did not display equivalent changes in serum cytokines during the study period. In most cases, the temporal relationship between the cytokine surge and the appearance of autoantibodies was reasonably close, considering the minimal interval of 3 months between sampling and given the cytokine surge most likely reflected innate immune and T-cell activation that preceded the evolution of autoantibodies, the dynamics of which may vary between individuals. Furthermore, in several cases, the cytokine surge was associated with a change in autoantibody specificity, suggesting an association with both primary and secondary antigenic responses. Timing in relation to the appearance of islet autoimmunity and before progression to clinical diabetes links the cytokine surge to disease pathogenesis.

Increased concentrations of serum cytokines have been reported in children before the onset of clinical type 1 diabetes^{11–13} but the cytokines were not mucosa-associated or studied in relation to seroconversion. However, in children with multiple islet autoantibodies close to diagnosis, Viisanen *et al.*¹⁴ observed an increase in circulating follicular T helper

cells, a major source of IL-21, a mucosa-associated cytokine. In the present study, serum IL-21 increased in 7/8 case children but in none of the controls. IL-21 is increased in inflammatory bowel disease in humans¹⁵, drives proliferation of NK cells, B cells and CD8⁺ T cells to kill virus-infected cells and, in concert with IL-23, promotes Th17 lineage differentiation¹⁶. IL-21 also activates production of IL-22¹⁷, which promotes intestinal epithelial integrity and is a key element of the innate immune response to enteric bacterial infection¹⁸. In the non-obese diabetic (NOD) mouse model of type 1 diabetes, IL-21 has key role in initiating islet autoimmunity by promoting the expansion of Th17 cells^{19,20}. A phase II clinical trial in adults with newly diagnosed type 1 diabetes of monoclonal antibody blockade of IL-21, together with liraglutide (a [glucagon-like peptide-1 receptor agonist](#) that promotes insulin secretion), found that the combination of agents, but not anti-IL-21 antibody alone, sustained endogenous insulin production and improved glucose metabolism²¹. Our finding lends support to the evidence that IL-21 is involved in the pathogenesis of islet autoimmunity.

IL-23 has a central role in intestinal inflammation, being critical for the generation and differentiation of Th17 cells that secrete IL-17F, IL-21, IL-22, and IFN- γ ²². IL-17F promotes inflammation in the intestine²³ and antibody blockade of IL-17 decreased the incidence of diabetes in the NOD mouse²⁴. Moreover, IL-17 immunity is upregulated in children before^{12,13} and at clinical diagnosis of type 1 diabetes^{25–28}. IL-25 (IL-17E) drives Th2 immunity and suppresses intestinal inflammation^{29,30}, and protects against diabetes in the NOD mouse²⁴. IL-33 is produced by intestinal and other mucosal epithelia, maintains epithelial barrier integrity, and activates innate and Th2 immunity in the intestine³¹. In summary, various lines of evidence link the cytokines we detected in seroconverters to the intestinal mucosa and the pathogenesis of type 1 diabetes. While they are not exclusive to the intestine, their detection in combination in significant concentrations in the systemic circulation suggests that other tissues, including the pancreas, are less likely sources of these cytokines.

Mucosa-related cytokines could signal the presence of infectious agents, e.g., viruses known to target both the small intestine and beta cells^{3–5}. Yeung *et al.*¹² did not find a relationship between serum cytokines and enterovirus detected by PCR in plasma or stool. However, others have reported that serum IL-17 and IL-23 are increased in children infected with enterovirus 71^{32,33} and are positively correlated with enterovirus IgG antibody titers in children with celiac disease and type 1 diabetes³⁴. We sought associations between the serum cytokines and parent-reported intestinal or respiratory infections, but none were found. Serial analysis of the gut virome, currently underway in ENDIA³⁵, will be a more sensitive and reliable means to determine if the serum cytokine surge reflects enteric virus infection.

The strength of this study is the matching of seroconverter cases and controls within a longitudinal sampling frame,

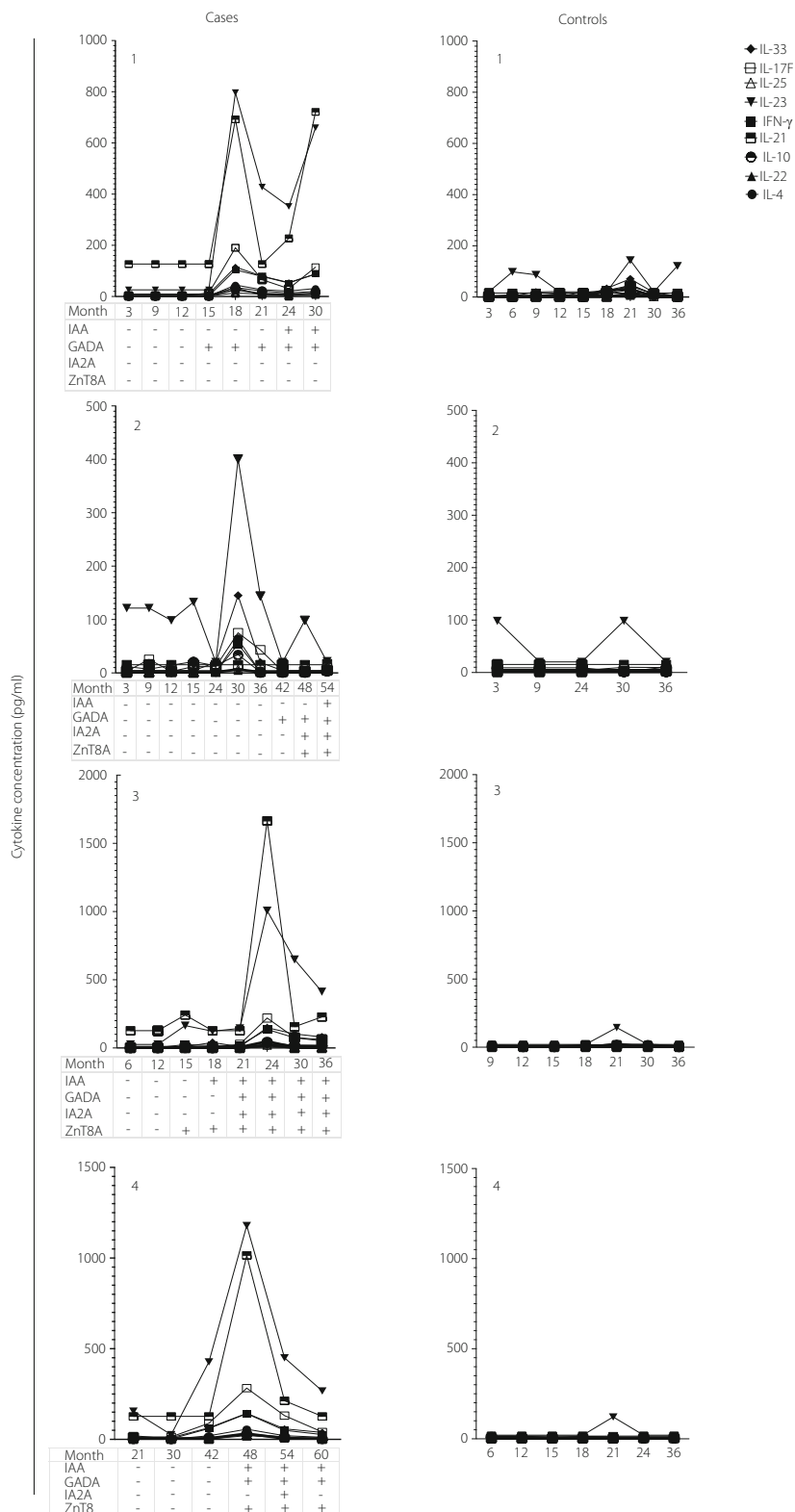
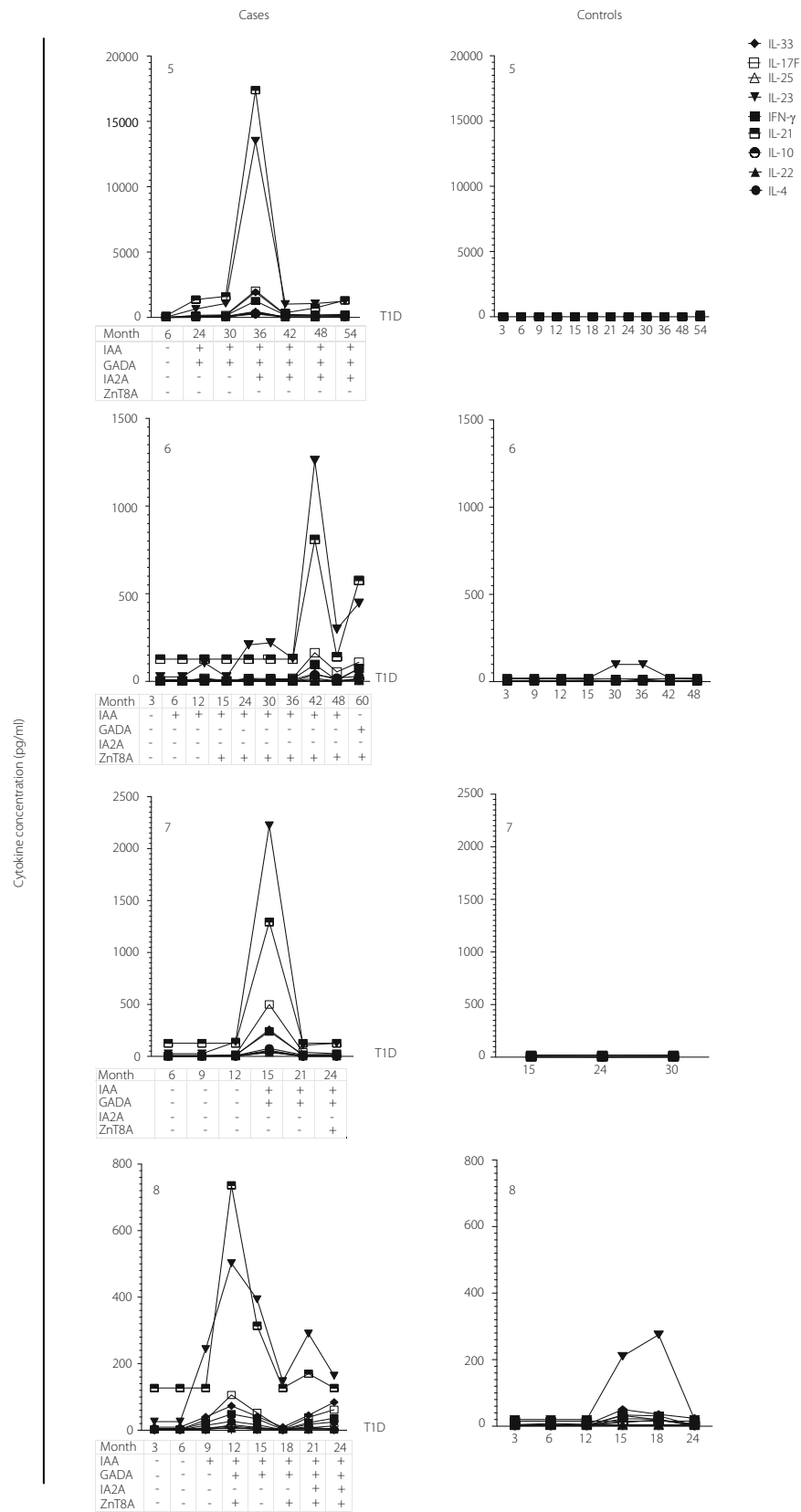


Figure 1 | Serum cytokine concentrations in children with islet autoimmunity and in age- and sex-matched controls. Below the x-axis is shown the absence (–) or presence (+) of islet autoantibodies to insulin (IAA), glutamic acid decarboxylase 65 (GADA), islet-associated antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) against age in months. Type 1 diabetes after the last time point indicates the diagnosis of clinical type 1 diabetes. Each figure set represents data from a matched case and control pair.



enabling the transient surges in mucosa-related cytokines to be captured in the seroconverters. The weakness of the study is that the criteria required to select the cases and controls limited the number of children available. If our findings are validated in a larger cohort, they would represent an important clue to the pathogenesis of type 1 diabetes.

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DISCLOSURE

The authors declare no conflicts of interest.

Approval of the research protocol: The ENDIA study was reviewed and approved by human research ethics committees at each clinical site, with the Women's and Children's Hospital Network Human Research Ethics Committee in Adelaide acting as the lead under the Australian National Mutual Acceptance Scheme (reference number HREC/16/WCHN/066). Conduct in Western Australia was approved by the Women and Newborn Health Service Ethics Committee (reference number RGS0000002639) and Child and Adolescent Health Service HREC (RGS0000002402). The research conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013).

Informed consent: Written informed consent was obtained by parents on behalf of themselves and the child.

Registry and the registration no. of the study/trial: The ENDIA study was registered with the Australia New Zealand Clinical Trials Registry July 16, 2013, under registration number ACTRN12613000794707.

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Serum cytokine concentrations in unmatched seronegative children